

Response to Luca L Fava and colleagues

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Reply to: **LL Fava et al** (in this issue)

We read with great interest the letter of Fava and colleagues, who challenge the conclusions of our article published in *EMBO Reports* (2013) [1]. Our work described a role for Beclin-1 in chromosome congression and outer kinetochore assembly. We demonstrated that this unexpected role of Beclin-1 is independent of its association with the PI3K-III complex and its well-documented role in autophagy. Our findings were based on knockdown of Beclin-1 using two different siRNAs and on rescue experiments. Additional biochemical and cellular biology experiments with native proteins also suggested a functional role for Beclin-1 in outer kinetochore assembly.

In this issue of *EMBO Reports*, Fava and colleagues suggest that Beclin-1 is dispensable for chromosome congression. They performed a set of experiments with two additional siRNAs targeting Beclin-1. One of them efficiently knocks down Beclin-1 at the protein level (siRNA Becn1-II); the second one only partially depletes Beclin-1 (siRNA Becn1-I). They report in their study that, in contrast to what we described, neither treatment affects mitotic progression in their live cell analysis. They found no delay in chromosome congression. However, by using one of our siRNA targeting Beclin-1 (named siRNA Becn1-ER in their report and siRNA Beclin-1 in our article), Fava *et al* confirmed our observations: Beclin-1 depletion affects the targeting of several outer kinetochore proteins, delays chromosome congression, and impairs progression through mitosis. Fava and colleagues conclude however that the phenotype is due to an off-target effect of our siRNA.

Despite the discrepancies with our results, we are confident in our data for several reasons:

1) A second siRNA (called siRNA Beclin-1 (#2)) in our article gave similar results to those obtained with siRNA Becn1-ER (see Supplementary Fig S1 in our paper by Frémont *et al* [1]).

2) A HeLa cell line stably expressing an (HA)-Beclin-1 construct resistant to the siRNA partially rescued the Beclin-1 depletion phenotype with respect to mitotic progression (the rescue of the other phenotypes ascribed to Beclin-1 was not assessed), arguing against an off-target effect of the siRNA. In examining this experiment in our paper, Fava *et al* remark: “Upon Beclin-1 knockdown, [however], both cell lines show a more than two-fold increase in G2/M cells when compared to the control. This actually documents that the Becn1-ER siRNA triggers the same relative increase in G2/M cells in both cell lines investigated and that this effect is not prevented by the expression of RNAi-resistant HA-Beclin 1.” However, we analyzed our results differently. We stated that ectopic expression of siRNA-resistant (HA)-Beclin-1 partially rescues (35%) the accumulation of 4n DNA content cells. Our conclusions were based on the calculation of the ratio of cells with 4n DNA content (G2/M cells) to cells with 2n DNA content cells (G0/G1 cells) in each experimental condition. Ratios obtained in cells depleted for Beclin-1 were compared to those obtained for siRNA control cells in each cell line. The effects of Beclin-1 depletion were thus compared between the two cell lines.

3) Most importantly, both our article and Fava’s report agree that treatment with siRNA Becn1-ER leads to an increase in the time elapsed between nuclear envelope

breakdown (NEBD) and anaphase. The severity of this phenotype depends on the concentration of transfected siRNA Becn1-ER in both studies (see Fig 2H in our article and Fig 1G in Fava’s report). However, because this phenotype did not correlate with the level of Beclin-1 detected by Western blot in Fava *et al*, they conclude that the siRNA Becn1-ER-induced phenotype is an off-target effect (Fig 1F). In contrast, in our paper, the concentration of transfected siRNA is indeed inversely proportional to the level of Beclin-1 detected by Western blot and to the number of correct mitotic events observed. Although knockdown of Beclin-1 with siRNA Becn1-II seems as efficient as the depletion induced by siRNA Becn1-ER transfection (see Supplementary Fig S1B), quantitative comparison of the efficiency of Beclin-1 depletion with these siRNAs (Becn1-II and Becn1-ER) at protein level should be performed head-to-head to reconcile these conflicting data.

4) Despite the research in databases (Blastn and RNA plex) and transcriptomic experiments, Fava *et al* could not identify a “smoking gun” off-target candidate that might explain the discrepancies between our paper and their report.

5) In addition to the mitotic progression phenotype of the siRNA treatment, we also showed that Beclin-1 associates with kinetochore microtubules, and forms discrete foci at the kinetochores of attached chromosomes. Moreover, we also identified a direct interaction between Beclin-1 and the outer kinetochore protein Zwint-1 (previously reported by Behrends and colleagues [2]), a component of KMN (KNL-1/Mis12/Ndc80) complex which is essential for kinetochore–microtubule interactions and chromosomes congression. These results are coherent with

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the phenotype of our siRNA studies and argue for a genuine role of Beclin-1 in chromosome congression.

Taken together, the collection of data and controls in our paper support a role for

Beclin-1 in mitosis progression. The report from Fava *et al* does reveal how siRNA treatment may have a widespread effect on cellular transcripts, but it does not address Beclin-1's function in kinetochore assembly.

References

1. Frémont S, Gérard A, Galloux M *et al* (2013) *EMBO Rep* 14: 364–372
2. Behrends C, Sowa ME, Gygi SP *et al* (2010) *Nature* 466: 68–76